Reference: *Biol. Bull.* **166**: 669–682. (December, 1984)

NUTRIENT TRANSLOCATION IN THE SEA STAR: WHOLE-BODY AND MICROAUTORADIOGRAPHY AFTER INGESTION OF RADIOLABELED LEUCINE AND PALMITIC ACID

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ABSTRACT

Using the sea star, *Asterias rubens*, whole-body autoradiography has been employed to follow the distribution and the pathways of translocation of both soluble and tissue-incorporated label derived from orally administered ^{14}C-labeled leucine or palmitic acid. Radioactivity remains localized predominantly in the stomach and pyloric caeca until sixteen days after ingestion. Labeling of the perivisceral coelomic cavity in regions close to the stomach shortly after ingestion points to initial displacement of ingested nutrients through the coelomic fluid and coelomocytes. After oral administration of labeled palmitic acid, distinct labeling of the gastric hemal tufts, axial organ, and aboral ring prior to labeling of the gonads also suggests the involvement of hemal tissue and surrounding perihemal coelomic sinuses in storage and translocation of substances needed for gamete nutrition.

Microautoradiography of gonad tissue reveals a rapid labeling of the walls of the genital coelomic sinus, the ground substance of the genital hemal sinus, and, after prolonged incubation, the germinal epithelium. Little or no label is incorporated into the outer sac of the gonad wall.

The results are discussed in terms of current knowledge on nutrient translocation in the sea star.

INTRODUCTION

Several physiological studies during the last two decades have investigated the pathways and mechanisms of nutrient translocation in the sea star. Various techniques have been employed to follow transfer of nutrients from digestive and storage organs (pyloric caeca) to peripheral areas of the body. Such techniques are generally based on oral administration of radiolabeled nutrients or non-nutritive tracers. Subsequent sampling from coelomic compartments possibly involved in translocation reveals rapid transport of small amounts of tracer from the digestive system to the perivisceral coelomic cavity (Ferguson, 1964a) and the (perihemal) axial sinus (Broertjes *et al.*, 1980b). The nature and the amount of radioactive substances in several internal organs at various times after ingestion of a radiolabeled precursor can be determined using extraction and analytical procedures (Oudejans and Rutten, 1982; Oudejans *et al.*, 1983). The results of these studies also indicate transfer through the coelomic cavity and a possible involvement of coelomocytes in lipid transport. By oral administration—or infusion into the axial sinus—of a vital dye, Broertjes and Posthuma (1978) and Broertjes *et al.* (1980a) demonstrated the possibility of translocation of substances from stomach to gonads through the hemal system; this system would be

Received 9 August 1984; accepted 19 September 1984.

operative especially in conveying high-molecular substances needed for vitellogenesis (Broertjes *et al.*, 1980b).

Up to now, autoradiography of orally administered radiolabeled nutrients in sea stars has been performed in three studies. Ferguson (1963) studied autoradiographs prepared from sections of the rays of *Asterias forbesi* fed with clams which had been injected with various radioactive organic compounds. He concluded that the perivisceral fluid and other body fluids were primarily responsible for nutrient translocation; no evidence indicating a transport role for the hemal system or coelomocytes was found. Broertjes *et al.* (1980b) demonstrated radioactivity in deeper layers of the digestive epithelium of the pyloric stomach and the associated hemal tissue of *Asterias rubens* after ingestion of radiolabeled leucine. During the preparation of this paper, a systematic study was published in which the distribution of radioactivity in digestive system, body wall, hemal organs, and gonads of *Echinaster* was delineated autoradiographically at various times after ingestion of radiolabeled amino acids (Ferguson, 1984); results were interpreted as indicative of a role of hemal tissue in translocation of nutritive substances to the gonads and parts of the tube feet.

Since the autoradiographic studies mentioned above have all been performed on Bouin-fixed paraffin sections of small pieces of specific organs, they do not provide information on the overall distribution of radiolabel throughout the asteroid body or on the distribution of labeled compounds that are lost during fixation and dehydration procedures (*e.g.*, amino acid not incorporated into proteinoid material). In the present study we employed whole-body autoradiography to follow the fate of ingested ^{14}C-labeled L-leucine and palmitic acid in *Asterias rubens*. This technique combines good resolution with the possibility of interpretation of the overall distribution in whole-body sections and allows localization of soluble compounds as it is applied to unfixed frozen tissue. Microautoradiography on semi-thin sections of gonad tissue was performed simultaneously in order to obtain more detailed information on the pathways of translocation of substances needed for gonad development.

Materials and Methods

Animals

Mature specimens of *Asterias rubens* were collected in the Dutch Wadden Sea. The animals were kept at natural daylength in a flow-through sea water system at 12°C and fed *ad libitum* with the sea mussel *Mytilus edulis*. Specimens with body weights from 70 to 100 g and arm-lengths of approximately 7 cm (measured from the center of the disc) were used. The experiments were performed during active gametogenesis in the period of December 1982 to February 1983.

Radioactive precursors

L-[^{14}C(U)]-leucine (13.1 GBq/mmol) and [^{14}C(U)]-palmitic acid (29.6 GBq/mmol) were obtained from New England Nuclear (Dreieich, W. Germany). L-leucine was dissolved in 3% NaCl and palmitic acid was dissolved in 3% NaCl containing 5% bovine serum albumin. Each animal received a final volume of 0.2 ml of precursor solution (total radioactivity 0.37 MBq = 10 μCi).

Experimental procedure

Sea stars were temporarily immobilized in a 1:1 mixture of 3% $MgCl_2$—blocking neuromuscular transmission—and sea water for 10 minutes. This procedure prevented the adambulacral spines from covering the peristome and thus facilitated access to the esophagus. Precursor was administered with a microsyringe fitted with a poly-

ethylene tube to prevent injury of the stomach. Sea stars were laid upside down and, after careful insertion of the tube into the esophagus, the precursor solution was injected very slowly into the stomach. Five minutes after removal of the syringe, the animals were put in fresh sea water. After 25 minutes, each sea star was placed separately in a large beaker, containing 1500 ml of fresh sea water which was aerated continuously. The temperature of the sea water was maintained at approximately 13°C. The animals were allowed to feed on specimens of *Mytilus edulis*.

Autoradiography

At 1.5, 6, and 24 hours, and at 4 and 16 days (in pilot experiments also at 4 hours and 6 days) after ingestion of the precursor, animals were immobilized in 3% $MgCl_2$ for 5 min. Animals were then frozen by submersion in hexane cooled with solid carbon dioxide (−75°C), wrapped in polyethylene bags, and stored in sealed boxes at −60°C up to 4 months. Other specimens were dissected after immobilization; small fragments of the gonads were fixed in 3% glutaraldehyde in 0.2 *M* sodium cacodylate buffer (pH 7), containing NaCl (30 mg/ml) and $CaCl_2$ (2 μg/ml), for 2 h at 4°C (Walker, 1979). Postfixation in 1% OsO_4 for 2 h at 4°C was followed by dehydration in ethanol and propylene oxide and embedding in Epon 812/Araldite 506.

For whole-body autoradiography, frozen animals were embedded in carboxymethylcellulose gel and sectioned in a LKB 2250 PMV cryomicrotome at −20°C. Sections of 50 μm thickness were taken parallel to the oral and aboral surface at intervals of 1 mm and picked up with transparent adhesive tape. For histological observations, sections were fixed in a 4% formaldehyde solution in 100% ethanol for 1 min at −20°C and stained with hematoxylin-eosin. For autoradiography, sections were freeze dried inside the cryostat, sprayed lightly with talcum powder, and placed on X-ray film (Agfa-Gevaert Structurix D-10). After exposure at room temperature for two weeks, contact prints of the developed negatives were made using one standard exposure time and paper quality to allow direct comparison of results. Controls for chemography were satisfactory.

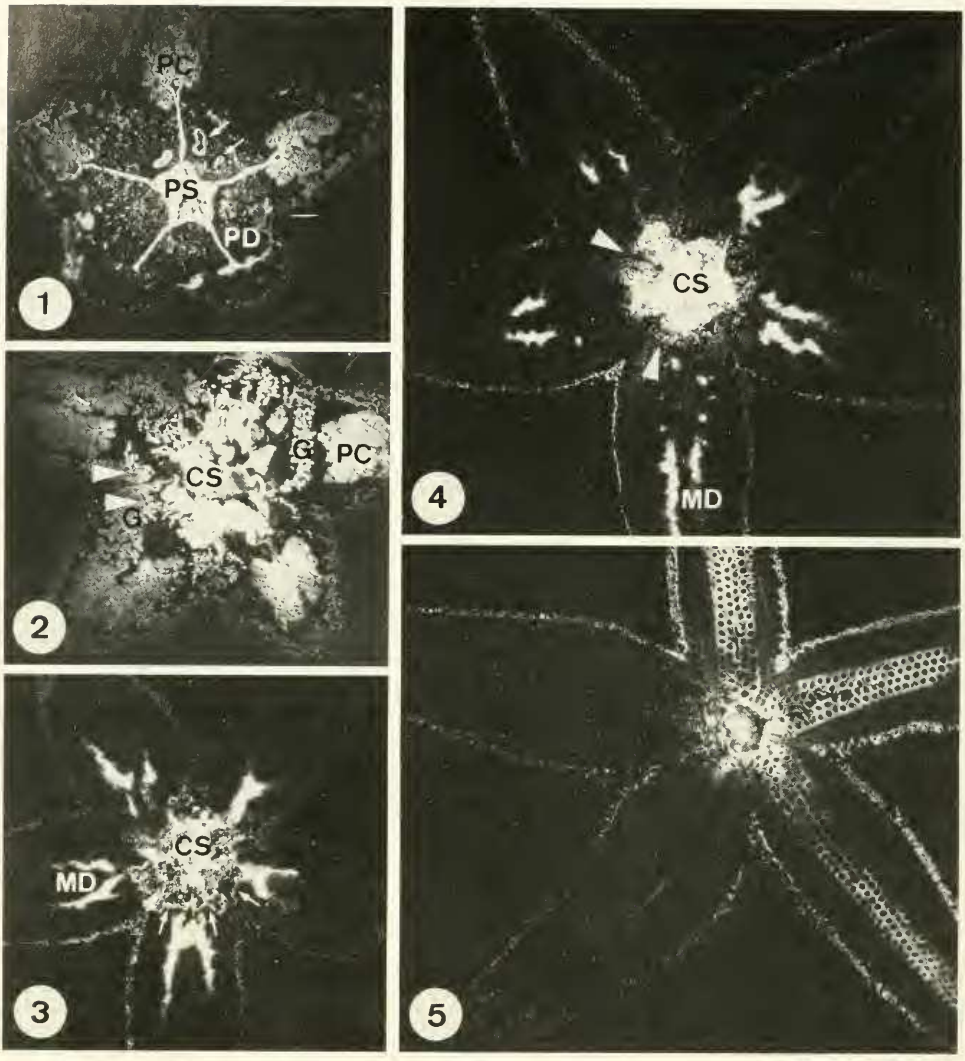
For microautoradiography, semi-thin sections (1 μm) of plastic embedded tissue were cut on a Reichert OMU 3 ultramicrotome. Sections were attached to gelatin coated slides by heating. Since pilot experiments revealed strong negative chemography, sections were stained first with Methylene-Blue/Azure II; slides were then coated by double immersion in 0.5% collodion in amyl acetate, and air dried for two days. The slides were dipped in Ilford K2 liquid nuclear emulsion (diluted 1:1 with 1% glycerol in distilled water), air dried, sealed in light-tight boxes containing a dessicant, and exposed at 5°C for 40 and 100 days. The micrographs presented in the next sections are made from preparations developed after 100 days.

For each incubation time and precursor used, one animal (sometimes two) was studied throughout from aboral to oral body wall by whole-body autoradiography, while gonad tissue from two to four animals (of both sexes when possible) was used for microautoradiography.

Results

Whole-body autoradiography after administration of radiolabeled leucine.

Digestive system. At all incubation times employed, the most prominent levels of tracer observed in whole-body sections are present in the walls of the cardiac and pyloric parts of the stomach and in the median ducts of the pyloric caeca (Figs. 1–10). The side lobes of the pyloric caeca reveal significant labeling only after short



FIGURES 1–10. Full-sized contact-autoradiographs of (parts of) whole-body sections (50 μm thickness) of specimens of *Asterias rubens*, frozen at timed intervals after oral administration of L-[^{14}C(U)]-leucine (0.37 MBq = 10 μCi per animal).

FIGURE 1. Animal sacrificed after one and a half hours. The roof of the pyloric stomach (PS), the five pyloric ducts (PD), and the walls of the rectal caeca (arrows) are distinctly labeled. Note diffuse labeling of the central region of the perivisceral coelomic cavity surrounding the stomach. PC: pyloric caeca.

FIGURE 2. Same animal as in Figure 1, section taken more orally. The folds of the cardiac stomach (CS) and the proximal parts of the pyloric caeca (PC) are strongly labeled. Gonads (G) are visible as highly lobulated strands of tissue and display radioactivity predominantly in regions close to the stomach (arrowheads).

FIGURE 3. Animal sacrificed after four hours. Cardiac stomach (CS) and median ducts (MD) of pyloric caeca are strongly labeled. Note diffuse radioactivity of the central region of the perivisceral coelomic cavity and narrow bands of tracer along the proximal parts of the peritoneal lining of the pyloric caeca (arrows).

FIGURE 4. Same animal as in Figure 3, section taken more orally to show radioactivity in cardiac stomach (CS) and median ducts (MD) of pyloric caeca. Note labeling of lobulated gonad tissue in regions close to the stomach (arrowheads).

FIGURE 5. Same animal as in Figure 3, section of ambulacral region. The body wall, podia (visible in cross-section as grouped circles), and mouth region are labeled.

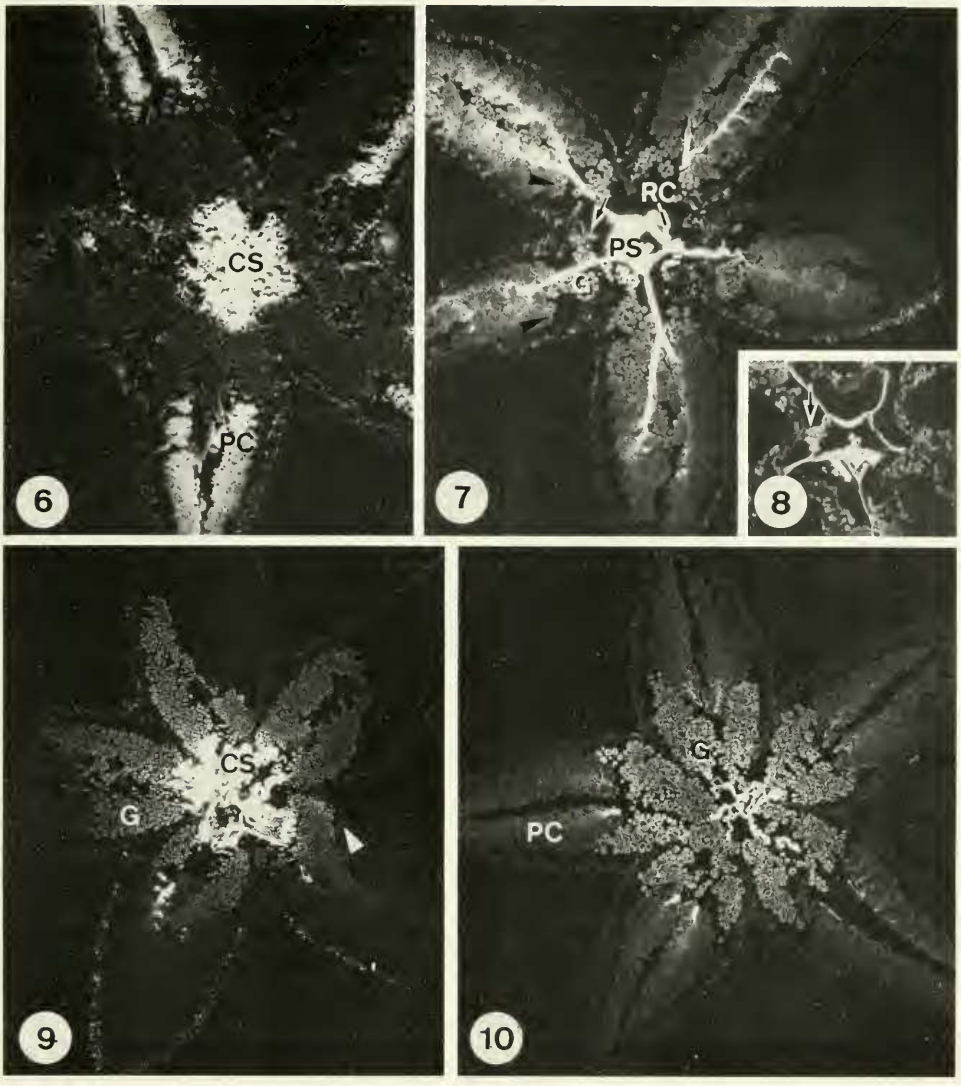


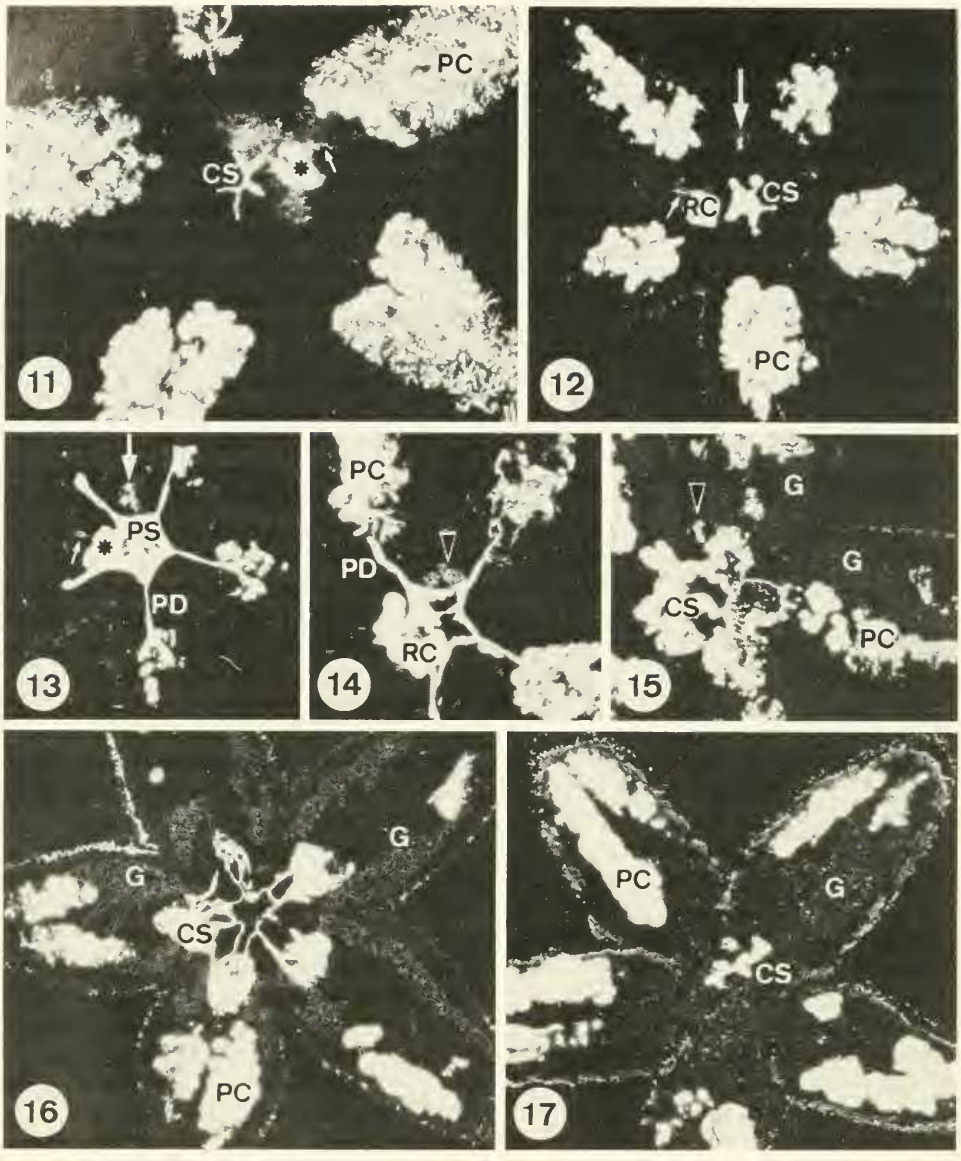
FIGURE 6. Animal sacrificed after six hours. Folds of cardiac stomach (CS) and side lobes of the pyloric caeca (PC) are labeled. The gonad tissue, present throughout the central area of the section, shows peripheral labeling.

FIGURE 7. Animal sacrificed after one day. The tissues of the pyloric stomach (PS), pyloric and median ducts (visible as broad white bands), and rectal caeca (RC) are strongly labeled. Moderate levels of radioactivity are present in the gonads (G). The side lobes of the pyloric caeca are slightly labeled. Note distinct narrow bands of radioactivity over the peritoneal lining of the pyloric caeca (black arrowheads). Arrow points to slightly labeled axial organ.

FIGURE 8. Same animal as in Figure 7, section taken more aborally to show labeling of gastric hemal tufts (arrow).

FIGURE 9. Animal sacrificed after one day (other animal as in Figs. 7, 8). The folds of the cardiac stomach (CS) are strongly labeled. Moderate levels of radioactivity are present in the gonads (G) with highest levels near the stomach. White arrowhead indicates labeling of peritoneal lining of pyloric caeca; side lobes are labeled only very little. Labeling of the walls of the ampullae can be observed in the upper and upper right arm.

FIGURE 10. Animal sacrificed after sixteen days. Folds of cardiac stomach (center) and gonads (G) are distinctly labeled. PC: pyloric caeca.



FIGURES 11–17. Full-sized contact autoradiographs of (parts of) whole-body sections (50 μm thickness) of specimens of *Asterias rubens*, frozen at timed intervals after oral administration of [^{14}C(U)]-palmitic acid (0.37 MBq = 10 μCi per animal).

FIGURE 11. Animal sacrificed after one and a half hours. The cardiac stomach (CS) and pyloric caeca (PC) are strongly labeled. Arrow indicates narrow band of radioactivity along the lining of a gonad. The central area of the coelomic activity is distinctly labeled (asterisk).

FIGURE 12. Animal sacrificed after six hours. High levels of radioactivity are present in cardiac stomach (CS), rectal caecum (RC), and pyloric caeca. Small arrow indicates tiny band of radioactivity in interbrachial septum; big arrow points to strongly labeled axial organ.

FIGURE 13. Same animal as in Figure 12, section taken more aborally. Note strong labeling of the roof of the pyloric stomach (PS), pyloric ducts (PD), and rectal caecum (asterisk). Small arrow indicates band of tracer in the interbrachial septum; big arrow indicates labeled gastric hemal tufts.

FIGURE 14. Animal sacrificed after one day. High levels of tracer are present in pyloric caeca (PC), pyloric ducts (PD), and rectal caeca (RC). Arrowhead indicates moderately labeled gastric hemal tufts.

FIGURE 15. Same animal as in Figure 14, section taken more orally to show labeling of axial organ (arrowhead) and folded cardiac stomach (CS). Gonads (G) are slightly labeled. PC: pyloric caeca.

periods of incubation (Figs. 2, 6); generally, levels of radioactivity in the side lobes are relatively low and do not noticeably increase during prolonged incubation (Figs. 7, 9, 10). At the site of the coelomic epithelium of the pyloric caeca, a sharp band of radioactivity is present after incubation times of four hours and more (Figs. 3, 7, 9). Shortly after feeding, this band is especially conspicuous in regions of the pyloric caeca that lie close to the stomach (Fig. 3).

The walls of the rectal caeca reveal significant amounts of tracer in all sections studied (*e.g.*, Figs. 1, 7).

Coelomic cavity and hemal tissues. Shortly after ingestion of labeled leucine, scattered labeling of the coelomic area surrounding the pyloric stomach is observed (Figs. 1, 3). The gastric hemal tufts are moderately labeled after one day (Fig. 8); radioactivity of these organs is less pronounced after prolonged incubation. Whereas some moderate labeling of the axial organ has been observed after one day (Fig. 7), distinct bands of radioactivity indicating the presence of tracer within the aboral ring have not been detected.

Gonads and body wall. Labeling of the gonads one and a half hours after ingestion is especially manifest in regions close to the stomach (Figs. 2, 4). After four hours, the gonads become labeled more intensely and more uniformly—although radioactivity remains localized mainly in the periphery of the gonad tissue—and ultimately may display levels of radioactivity higher than those observed in the side lobes of the pyloric caeca (Figs. 7, 9, 10).

The body wall is labeled relatively little at all incubation times. High levels of tracer are present in the ambulacral tissues and tube feet (Fig. 5); these levels increase during prolonged incubation.

Whole-body autoradiography after ingestion of radiolabeled palmitic acid

Digestive system. One and a half hours after ingestion, tracer is seen dispersed throughout the pyloric caeca (Fig. 11). After prolonged incubation, the most prominent levels of tracer observed in whole-body sections are present in the pyloric caeca. At all incubation times, very pronounced levels of tracer are also present in the walls of the pyloric and cardiac parts of the stomach (Figs. 11–17).

The walls of the rectal caeca remain distinctly labeled until 16 days after administration. The rectal lumen is also strongly labeled after shorter periods of incubation (Figs. 12, 13).

Coelomic cavity and hemal organs. One and a half hours after ingestion, conspicuous amounts of tracer are present in the central area of the coelomic cavity near the cardiac and pyloric parts of the stomach (Fig. 11). The gastric hemal tufts are distinctly labeled after 6 to 24 hours (Figs. 13, 14). During prolonged incubation, the amounts of tracer present in the gastric hemal tufts seem to decrease; the same applies to the axial organ (Figs. 12, 15). Six hours after ingestion, distinct bands of radioactivity along the interbrachial septum indicate labeling of the aboral ring (Figs. 12, 13).

Gonads and body wall. After one and a half hours, distinct narrow bands of label can be distinguished along the margins of the gonads in regions close to the stomach (Fig. 11). After six hours to one day, gonads are slightly labeled (Fig. 15). Levels of

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FIGURE 16. Animal sacrificed after four days. Highest levels of radioactivity are present in pyloric caeca (PC) and walls of cardiac stomach (CS). Sharp bands of radioactivity are observed over the lining of the gonads (G).

FIGURE 17. Animal sacrificed after sixteen days. Note strong labeling of the pyloric caeca (PC), cardiac stomach (CS), and body wall. The periphery of the gonads (G) is sharply labeled.

radioactivity slightly increase during prolonged incubation but stay relatively low (Figs. 16, 17); label remains distinctly localized in the gonadal periphery.

The body wall is slightly labeled after all incubation times employed. The tube feet and ambulacral tissues display relatively low levels of tracer after incubation times of six hours and longer.

Microautoradiography of gonad tissue

Only very low levels of label from ^{14}C-leucine are observed in semi-thin sections of ovaries. Figure 19 shows labeling of the ooplasm of fully vitellogenic oocytes six hours after ingestion of leucine; regions of the ooplasm near the gonad wall reveal highest levels of radioactivity. Little radioactivity is present in the gonad wall. In sections of ovaries fixed after other incubation times, levels of radioactivity are too low to be discernable from background activity.

In testes, label from leucine is generally much easier to observe. Figure 18 (arrows) shows small patches of silver grains above the myoepithelial lining of the genital coelomic sinus one and a half hour after ingestion of leucine; no radioactivity is present in spermatogenic columns, genital hemal sinus, or wall of this testis. After prolonged incubation, increasing levels of radioactivity are observed in the ground substance of the genital hemal sinus (Fig. 21), whereas the outer connective tissue layer and visceral peritoneum ("outer sac") are only slightly labeled (Figs. 20–24). Label becomes incorporated in basal regions of the spermatogenic columns initially (Fig. 20) and spreads throughout the columns during prolonged incubation (Figs. 21, 22); after 16 days, the chains of spermatids at the tip of the columns are also labeled (Fig. 23).

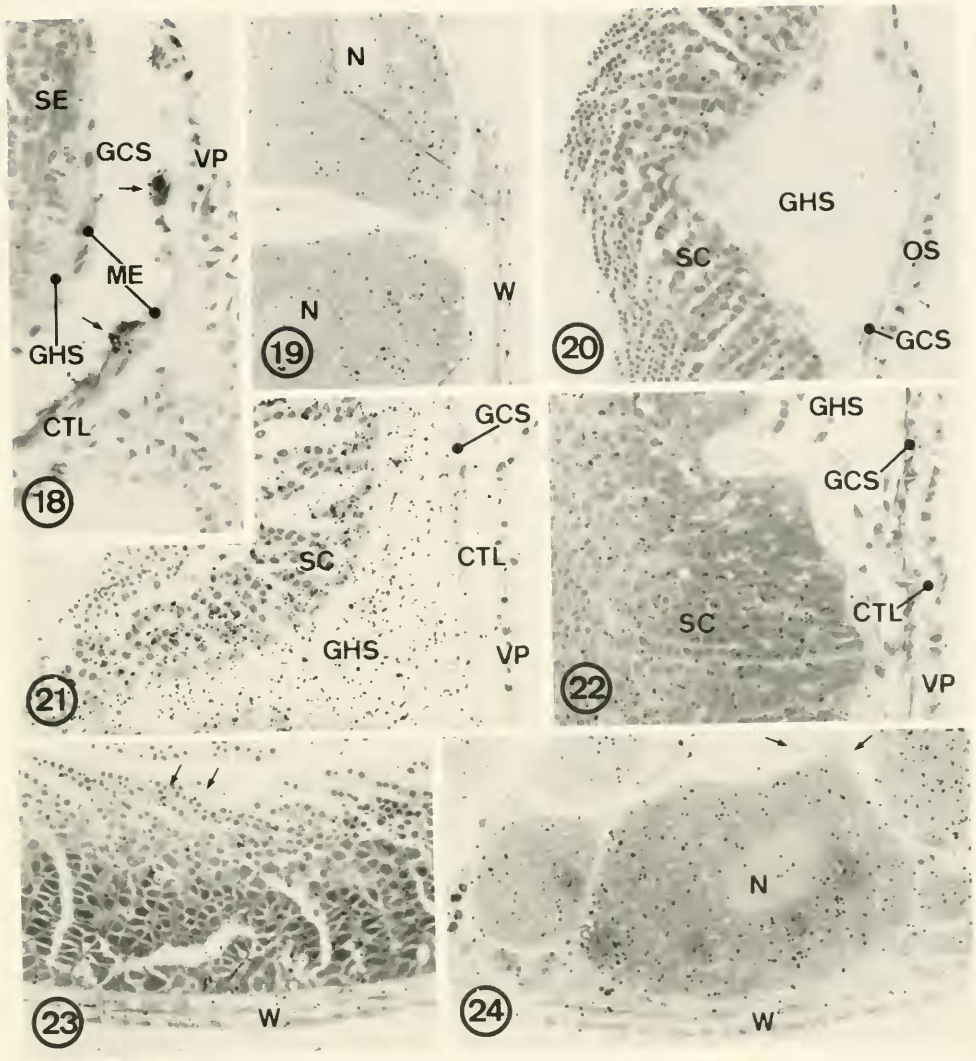
After administration of radiolabeled palmitic acid, no radioactivity clearly discernable from background levels is observed in testes and ovaries after incubation times of one and a half hours to one day. For longer periods of incubation, only sections of ovaries were available; in these sections, distinct labeling was observed not earlier than 16 days after ingestion, with highest levels of radioactivity present throughout the ooplasm and little or no radioactivity in the gonad wall (Fig. 24). No distinct labeling of follicle cells (Fig. 24, arrows) or coelomocytes, frequently attached to the peritoneum, has been observed.

Discussion

Source organs: digestive system

The observed distribution of radiolabel in "source" organs (stomach and pyloric caeca), "transport" organs (hemal tissues and coelomic cavities) and "recipient" organs (gonads, ambulacral tissues, and body wall) reveals several interesting features which demand comparison with earlier autoradiographic and analytical studies.

Little radioactivity could be recovered in isolated pyloric caeca, ovaries, coelomic fluid, and coelomocytes of *Asterias rubens* after oral administration of radiolabeled trioleylglycerol, leucine, and glucose (Oudejans and Rutten, 1982; Oudejans *et al.*, 1983). This is in agreement with the present observations revealing high levels of radioactivity—especially after administration of leucine—predominantly present in the tissues of the pyloric and cardiac parts of the stomach. To a lesser degree, the same holds for palmitic acid although label from this compound is distributed throughout the pyloric caeca much easier than the label from leucine; apparently, absorption of dietary fatty acid by the major storage organs of *Asterias rubens* is a fast process.



FIGURES 18–24. Stained autoradiographs of 1 μm sections of glutaraldehyde/osmium tetroxide-fixed gonad tissue of specimens of *Asterias rubens*, dissected at timed intervals after oral administration of L-[^{14}C(U)]-leucine or [^{14}C(U)]-palmitic acid (0.37 MBq = 10 μCi per animal). All magnifications 350×.

FIGURE 18. Testis, one and a half hours after ingestion of leucine. Arrows indicate labeling of the outer myoepithelial lining (ME) of the genital coelomic sinus (GCS). SE: spermatogenic epithelium; GHS: genital hemal sinus; CTL: connective tissue layer of outer sac; VP: visceral peritoneum.

FIGURE 19. Ovary, six hours after ingestion of leucine. Two vitellogenic oocytes show radioactivity, predominantly near the wall (W) of the ovary. N: nuclei of oocytes.

FIGURE 20. Testis, six hours after ingestion of leucine. Some radiolabel is present in basal regions of the spermatogenic columns (SC). Little or no tracer is present in the outer sac (OS) or in the swollen genital hemal sinus (GHS) and the amoeboid cells contained. GCS: compressed genital coelomic sinus.

FIGURE 21. Testis, one day after ingestion of leucine. Prominent levels of tracer are present throughout the spermatogenic columns (SC) and the ground substance of the genital hemal sinus (GSH). GCS: compressed genital coelomic sinus; CTL: connective tissue layer of outer sac; VP: visceral peritoneum.

FIGURE 22. Testis, four days after ingestion of leucine. Label is present throughout the spermatogenic columns (SC). GHS: genital hemal sinus with amoeboid cells; GCS: compressed genital coelomic sinus with myoepithelial lining; CTL: connective tissue layer of outer sac; VP: visceral peritoneum.

FIGURE 23. Testis, sixteen days after ingestion of leucine. Label, which is present mainly in basal regions of the spermatogenic columns, can also be observed in the wall (W) and in spermatids (arrows) at the tips of the columns.

FIGURE 24. Ovary, sixteen days after ingestion of palmitic acid. Distinct levels of radioactivity are present in the ooplasm of vitellogenic oocytes. N: oocyte nucleus; W: wall of ovary. Arrows indicate follicle cells.

Autoradiographs of paraffin sections of *Echinaster graminicolus* fed labeled amino acids (Ferguson, 1984) also revealed high levels of radioactivity in cardiac stomach and pyloric caeca, indicating substantial incorporation of amino acid into insoluble proteinoid material in these organs. Such incorporation was also demonstrated biochemically for the pyloric caeca of *Asterias rubens* (Oudejans *et al.*, 1983). Broertjes *et al.* (1980b) studied autoradiographs of the strongly folded roof of the pyloric stomach of the same species after oral administration of radiolabeled leucine and observed a progression of relatively low levels—as compared with the results from the present study—of insoluble radiolabel from apical regions of the digestive epithelium into deeper layers of the pyloric stomach tissue. Little radioactivity was observed in paraffin sections of the pyloric stomach of *Echinaster* fed radiolabeled amino acids (Ferguson, 1984). In contrast with these two studies, the present investigation reveals high levels of radioactivity in frozen sections of the roof and side walls of the pyloric stomach after administration of leucine, suggesting that amino acids, absorbed in these regions, are relatively little incorporated into tissue-protein—as demonstrated by paraffin section autoradiography—and may therefore be transferred directly to the adjacent hemal tissues (gastric hemal tufts) or coelomic fluid.

Using γ-camera equipment, Broertjes *et al.* (1982) could follow the fate of ingested ^{125}I-labeled mussel protein *in vivo*. Results of these experiments lent support to a model postulated earlier (Broertjes *et al.*, 1980a, b). According to this model, materials stored temporarily in the pyloric caeca are released into the caecal lumen and moved by mucoid-ciliary action into the direction of the folded roof of the pyloric stomach where they are absorbed by the digestive epithelium and pass through to the gastric hemal tufts. The present study does not give evidence for the release of stored nutrients into the caecal lumen; according to Broertjes *et al.* (1982) it would however take about one month after initial storage of radiolabeled food before such release occurs.

Distinct labeling of the coelomic lining of the pyloric caeca shortly after ingestion of radiolabeled leucine demonstrates that the coelomic epithelium absorbs nutrients from the coelomic fluid as was demonstrated also *in vitro* by Ferguson (1964b, 1968). The observed phenomenon does not necessarily prove net uptake of amino acid from the coelomic fluid; simultaneously with the observed uptake of labeled material, a more substantial release—due to the steep concentration gradient—may occur (Ferguson, 1982) which can not be monitored inasmuch as it concerns unlabeled compounds from already existing caecal depots. Up to now, such release has only been demonstrated using pyloric caeca *in vitro* submersed in nutrient depleted sea water (Ferguson, 1964b).

The observed high levels of tracer in the rectal caeca are in agreement with information from morphological studies indicating a high power of absorption of these organs (Jangoux, 1972, 1976).

Transport organs: coelomic cavity and hemal tissues

Shortly after ingestion of labeled leucine or palmitic acid, substantial amounts of tracer are present in the central area of the perivisceral coelomic cavity near the stomach. This unique observation, accomplished by the special features of the technique employed, conforms to the results of earlier studies (Ferguson, 1964a; Oudejans *et al.*, 1983) which show the rapid exchange of molecules between digestive system and coelomic fluid. Stained whole-body sections show eosinophilia in these coelomic regions, indicating the presence of coelomocytes. These amoeboid cells probably function in some transfer of fatty acids as it is unlikely that substantial amounts of these compounds are dissolved in the coelomic fluid. The high specific radioactivity of coelomocyte lipids, observed after oral administration of labeled trioleylglycerol, may support this view (Oudejans and Rutten, 1982).

Although initial distribution of ingested nutrients through the coelomic fluid and coelomocytes—also indicated by the rapid labeling along the peritoneal lining of gonads and pyloric caeca in regions close to the stomach—seems evident from the results presented here, the turnover rates of coelomic fluid compounds are by far insufficient to meet the demands of the developing ovaries during vitellogenesis (Beijnink *et al.*, 1984a). Alternatively, several authors have suggested involvement of the hemal system in transfer of materials from digestive organs to gonads and other areas of the body (reviews: Ferguson, 1982; Walker, 1979, 1982). Autoradiographic data, interpreted in favor of this hypothesis, were given by Ferguson (1970) who injected radiolabeled amino acids into the coelomic cavity of *Echinaster* and observed a marked accumulation of tracer in the radial strands of hemal tissue of the ambulacral regions. Significantly, label appeared simultaneously at different sites throughout the hemal strands; no progression of label from possible sites of origin towards sites of utilization was observed. Recently, Ferguson (1984) published an autoradiographic study indicating a progressive incorporation—from disc to gonads—of radiolabel derived from ingested amino acids into the ground substance of the aboral hemal ring and of the genital hemal sinuses. Again, it should be emphasized that these studies primarily demonstrate the incorporation of amino acid into insoluble material; thus, they are more indicative of synthesis or deposition of proteinoid material within hemal tissue rather than of translocation of nutrients through hemal tissue. In fact, it is unlikely that displacement of the mucoid hemal ground substance through the diminuitive hemal sinuses could occur at rates high enough to account for the prominent levels of radioactivity observed in the genital hemal sinuses 24 hours after ingestion of labeled leucine (Fig. 21). Alternatively, the hemal tissues might function as storage reservoirs within the surrounding perihemal coelomic sinuses which would then be primarily responsible for translocation; circulation within these fluid-filled coelomic channels is easily maintained by movement of the flagella of the coelomic lining (Walker, 1979). Such a storage function of hemal tissue was proposed earlier for species of three echinoderm classes (Grimmer and Holland, 1979; Walker, 1979; Ferguson, 1984; Jackson and Fontaine, 1984).

Up to now, no autoradiographic experiments designed to elucidate pathways of translocation of water-insoluble lipid compounds have been performed on echinoderm species. This study provides some evidence for the involvement of hemal tissues or enclosing coelomic sinuses in translocation of fatty acids: A very pronounced labeling of the gastric hemal tufts, the axial organ, and, presumably, the aboral ring is observed shortly after ingestion of palmitic acid. The subsequent decrease of levels of radioactivity in these hemal organs prior to the increase of radioactivity in the gonads may suggest the displacement of radiolabel through hemal tissue, or more likely, as argued above, the progressive temporary incorporation of label into hemal tissue from the surrounding (perihemal) coelomic cavities prior to release in the opposite direction.

Recipient organs: body wall and gonads

The body wall and associated ambulacral tissues show distinct levels of radioactivity shortly after ingestion of labeled leucine and, to a much lesser degree, of palmitic acid. Although several changes of fresh sea water have been employed during initial incubation, significant amounts of labeled compounds may have been present in the sea water due to leakage of precursor out of the esophagus. Therefore, the observed radioactivity of the body wall may be partly the result of epidermal uptake, a process well established in echinoderms (review: Bamford, 1982). As far as labeling of the body wall results from internal supplies, translocation through the coelomic fluid is probably involved.

Gonads display prominent levels of radioactivity at relatively short intervals after administration of labeled leucine. Biochemical analysis (Oudejans *et al.,* 1983) revealed the labeling of proteins and amino acids in the ovaries of *Asterias rubens* to be about equal one day after ingestion of labeled leucine. Microautoradiography also shows a substantial incorporation of label from leucine into insoluble (proteinoid) material in both testes and ovaries. Radiolabel derived from palmitic acid is conveyed apparently much more slowly to the gonads. While label from ingested trioleylglycerol after short periods of incubation (one hour to one day) could be recovered primarily in the triacylglycerol fraction after thin-layer chromatography of total lipids from sea star ovaries (Oudejans and Rutten, 1982), microautoradiography (Fig. 24) reveals that label from fatty acid is incorporated into compounds of the ooplasm that are not lost during dehydration in ethanol and propylene oxide [*e.g.* (glyco-)lipoproteins].

In the previous paragraphs, we have discussed the evidence for translocation either through the perivisceral coelomic cavity or through the (peri-)hemal system. As mentioned above, whole-body autoradiographs with gonad tissue show labeling patterns primarily supporting the first possibility; also, autoradiographic studies have demonstrated the penetration of thymidine, injected intra-coelomically (Walker, 1980; Van der Plas *et al.,* 1983) and adenine, applied *in vitro* (Toole *et al.,* 1974), into deeper layers of asteroid gonads. No patterns suggesting the dispersion of labeled substances throughout the gonads, away from the points of attachment to the body wall and the genital branches of the aboral ring, have been observed in whole-body autoradiographs. Analysis of the microautoradiographs, however, leads to other conclusions with regard to routes of translocation: remarkably, no label from leucine is incorporated into tissues of the outer sac of the gonad wall in sections where substantial amounts of tracer are observed in the germinal cells and/or the genital hemal sinus. This implies that nutrients such as leucine either pass from the coelomic fluid through the outer sac without being incorporated into tissue proteins, or more likely reach the inner sac by translocation through the genital coelomic and hemal sinuses. If one accepts the improbability of rapid translocation through the hemal sinuses, the coelomic sinuses of the aboral ring and gonad wall thus may perform crucial functions in short term nutrient transport towards the gonads. This view seems to be supported by the observed labeling of the myoepithelial lining of the genital coelomic sinus one and a half hours after ingestion of labeled leucine (Fig. 18, arrows). Of course, radiolabel within this fluid-filled sinus cannot be demonstrated with the microautoradiographic technique employed.

This study demonstrates a substantial incorporation of label from leucine and—to a lesser degree and only after prolonged incubation—from palmitic acid into the ground substance of the genital hemal sinus. Similar observations were made recently by Ferguson (1984) who demonstrated labeling of the genital hemal sinuses in spawned gonads of *Echinaster* fed radiolabeled amino acids. Apparently, both in true vitellogenic or spermatogenic stages, as studied in the present investigation, and in agametogenic stages, as studied by Ferguson (1984), incorporation of materials into hemal ground substance occurs. Histological and ultrastructural studies (Schoenmakers *et al.,* 1981; Beijnink *et al.,* 1984b) indicate that components of this ground substance are conveyed ultimately to vitellogenic oocytes; a recent immunocytochemical study demonstrates a close relationship between yolk glycolipoprotein and hemal ground substance (Beijnink *et al.,* 1984c).

Inasmuch as hemal sinuses are not recipient organs but transport organs, prominent levels of tracer within these sinuses point to high turnover rates, and, thus, to high rates of utilization of hemal ground substance. How incorporation of label from amino acids into proteinoid components of the virtually acellular hemal ground

substance (*e.g.*, Fig. 21) can be achieved, is only one of several intriguing problems that remain to be solved in order to better understand the complex mechanisms of nutrient digestion, translocation, and utilization in echinoderms.

ACKNOWLEDGMENTS

We are much indebted to Dr. C. J. A. Van den Hamer of the Radiochemistry Department of the Interuniversity Reactor Institute at Delft (The Netherlands) for the facilities offered and to Mr. L. Prinsen for his invaluable aid and stimulating enthusiasm during whole-body cryotomy. The assistance of Mrs. G. G. Oostveen in the microautoradiography procedures is gratefully acknowledged.

This investigation was supported by the Foundation for Fundamental Biological Research (BION) which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO).

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